Increased Proteolysis

An Effect of Increases in Plasma Cortisol within the Physiologic Range

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Abstract. Prolonged exposure to glucocorticoids in pharmacologic amounts results in muscle wasting, but whether changes in plasma cortisol within the physiologic range affect amino acid and protein metabolism in man has not been determined. To determine whether a physiologic increase in plasma cortisol increases proteolysis and the *de novo* synthesis of alanine, seven normal subjects were studied on two occasions during an 8-h infusion of either hydrocortisone sodium succinate (2 μg/kg · min) or saline. The rate of appearance (Ra) of leucine and alanine were estimated using [$^2H_3$]leucine and [$^2H_3$]alanine. In addition, the Ra of leucine nitrogen and the rate of transfer of leucine nitrogen to alanine were estimated using [^{15}N]leucine.

Plasma cortisol increased (10±1 to 42±4 μg/dl) during cortisol infusion and decreased (14±2 to 10±2 μg/dl) during saline infusion. No change was observed in plasma insulin, C-peptide, or glucagon during either saline or cortisol infusion. Plasma leucine concentration increased more ($P < 0.05$) during cortisol infusion (120±1 to 203±21 μM) than saline (118±8 to 154±4 μM) as a result of a greater ($P < 0.01$) increase in its Ra during cortisol infusion (1.47±0.08 to 1.81±0.08 μmol/kg · min for cortisol vs. 1.50±0.08 to 1.57±0.09 μmol/kg · min). Leucine nitrogen Ra increased ($P < 0.01$) from 2.35±0.12 to 3.46±0.24 μmol/kg · min, but less so ($P < 0.05$) during saline infusion (2.43±0.17 to 2.84±0.15 μmol/kg · min, $P < 0.01$). Alanine Ra increased ($P < 0.05$) during cortisol infusion but remained constant during saline infusion. During cortisol, but not during saline infusion, the rate and percentage of leucine nitrogen going to alanine increased ($P < 0.05$).

Thus, an increase in plasma cortisol within the physiologic range increases proteolysis and the *de novo* synthesis of alanine, a potential gluconeogenic substrate. Therefore, physiologic changes in plasma cortisol play a role in the regulation of whole body protein and amino acid metabolism in man.

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Introduction

Prolonged administration of pharmacologic amounts of glucocorticoids in man causes muscle wasting (1). Both stimulation of proteolysis and inhibition of protein synthesis have been implicated in this loss of body protein. The proteolytic effect of pharmacologic amounts of glucocorticoids on skeletal muscle is evidenced by increases in (*a*) efflux of amino acids from incubated rat muscle (2–4); (*b*) plasma amino acid concentrations in eviscerated animal preparations (5, 6); (*c*) plasma urea nitrogen (2); (*d*) muscle dipeptidase activity (7); and (*e*) 3-methylhistidine excretion (8). In contrast, no significant increase in protein degradation was observed in rat muscle following several days of high dose in vivo corticosterone exposure (9–11). Inhibition of protein synthesis also has been invoked to explain the protein catabolic state induced by steroids on the basis of decreased incorporation of amino acids into rat muscle following glucocorticoid treatment (9–12).

In man, increases in urinary nitrogen excretion and plasma concentrations of alanine and glutamine are observed only after 24–48 h of exposure to pharmacologic doses of glucocorticosteroids (13, 14). These data suggest that several days may be required before evidence of a glucocorticoid effect on amino acid metabolism can be seen in man. Whether short-term changes in plasma cortisol concentration within the physiologic range affect protein metabolism in man has not been determined. Infusion of cortisol to achieve high physiologic concentrations increases the plasma concentrations of phenylalanine and the

branched-chain amino acids, leucine, isoleucine, and valine (15). An increase in the plasma concentration of these essential amino acids in the postabsorptive state could result from either increased proteolysis or decreased utilization (oxidation and/or protein synthesis). Since the branched-chain amino acids are an important nitrogen source for synthesis of the potential gluconeogenic substrate alanine in vivo (16–18), cortisol stimulation of proteolysis could increase alanine availability via *de novo* synthesis, as well as directly from protein. The present studies were designed to determine if increases of plasma cortisol within the physiologic range could increase the rate of appearance of leucine, an indicator of whole body proteolysis, and, if so, to determine the effect of increased availability of leucine on the *de novo* synthesis of alanine.

Methods

Subjects. Informed consent was obtained from seven healthy adult volunteers between the ages of 23 and 38 yr. All were within 10% of ideal body weight (Metropolitan Life Insurance Table) and none had a family history of diabetes mellitus. The subjects had consumed a weight-maintaining diet containing at least 200 g of carbohydrate for a minimum of 3 d before study. All volunteers were admitted to the Clinical Study Unit on the afternoon before study and were provided a 600-cal meal at 1800 h. On the evening before study, an 18-gauge plastic catheter was inserted into a forearm vein for isotope infusion.

Experimental design. Each subject was studied on two occasions separated by at least 2 wk, once during infusion of hydrocortisone sodium succinate (2 μg/kg · min) beginning at 0 time, and on a second occasion during saline infusion. At −8 h, a constant infusion of L-[^{15}N]leucine was begun at a rate (0.14 μmol/kg · min) to enrich the circulating plasma pool of leucine to ~5 mol% and was continued to the end of the study. Isotopic equilibration of ^{15}N into the alanine pool has been demonstrated to occur in this period of time (18). On the morning of study, a primed constant infusion of L-[$^{2}H_3$]leucine (0.04 μmol/kg · min), L-[$^{2}H_3$]alanine (0.15 μmol/kg · min), and [^{3}H]glucose (3,800 dpm/kg · min) was begun at −4 h and continued throughout the study. At zero time (1100 h), an infusion of either saline or cortisol (2 μg/kg · min) was begun and continued throughout the study period (hour 8).

Arterialized venous blood was obtained from a 19-gauge scalp vein needle placed retrograde in the contralateral hand vein (19, 20). Blood samples were obtained at −2, −1.5, −1, −0.5, 0, 0.5, and 1 h, and subsequently at 1-h intervals through hour 8.

Approximately 17 ml of blood were drawn at each sampling and aliquoted in the following fashion: 5 ml were added to a tube containing EDTA and benzamadine for assay of glucagon, insulin, C-peptide, and cortisol; 10 ml were added to a tube containing sodium heparin for amino acid and substrate analysis; and 2 ml were added to a tube containing sodium fluoride for determination of glucose and glucose specific activity. All tubes were placed on ice, centrifuged at 4°C, and the plasma stored at −70°C until assay. Statistical analyses were carried out using a paired *t* test.

Analytical methods. L-[^{15}N]Leucine, L-[5,5,5-$^{2}H_3$]leucine, and L-[3,3,3-$^{2}H_3$]alanine (Merck, Sharp & Dohme, Isotope Division, Montreal, Canada) were determined to be pyrogen-free and >99% pure. Sterile, pyrogen-free D-[3-^{3}H]- and D-[2-^{3}H]glucose were obtained from New England Nuclear (Boston, MA).

Plasma leucine concentrations were determined by gas chromatography/mass spectrometry (GC/MS)[1] utilizing [$^{2}H_7$] leucine as an internal standard (21). Infusate amino acid concentrations were determined by ion exchange chromatography (Beckman 119 CL Autoanalyzer, Beckman Instruments, Inc., Fullerton, CA) (22). Plasma alanine, D-β-hydroxybutyrate, acetoacetate, lactate, and pyruvate were determined by microfluorometric enzymatic techniques (23–25). Plasma insulin (26), glucagon (27), and C-peptide (28) were measured by radioimmunoassay. Plasma cortisol was determined by protein-binding assay (29) and plasma FFA were determined colorimetrically (30).

Plasma glucose was measured using a YSI glucose analyzer (Yellow Springs Instrument Co., Yellow Springs, OH) and [^{3}H]glucose radioactivity was determined by the method of Dunn et al. (31). The metabolic clearance rate for glucose was calculated by dividing the rate of disappearance by the plasma concentration. Isotopic enrichment in plasma leucine and alanine was determined by GC/MS as previously described (17, 32) using the trimethylsilyl derivatives and a 2 m × 2-mm 3% OV 11 (Supelco, Inc., Bellefonte, PA) packed column interfaced by jet separator to a 5985B Hewlett Packard GC/MS (Hewlett-Packard Co., Palo Alto, CA). Moles percent enrichment of [^{15}N]alanine, [$^{2}H_3$]alanine, [^{15}N]leucine, [$^{2}H_3$]leucine, and [$^{2}H_7$]leucine were calculated by comparing the peak height ratio of the ions of interest from the plasma samples with those obtained on the same day from a standard curve of known enrichment. Actual rates of stable isotope infusion were determined as the product of the infusate amino acid concentration (micromoles per milliliter), isotope enrichment, and infusion rate (milliliter per minute).

Apparent rates of glucose appearance (Ra) and disappearance (Rd) were calculated from the equations of Steele (33), modified for nonsteady-state conditions (34). Apparent rates of appearance (Ra) and disappearance (Rd) for alanine and leucine were determined using Steele's equations for nonsteady-state conditions adapted for stable isotopic tracers (18, 35). The assumptions and equations for estimating Ra, Rd, and nitrogen exchange used have been previously described (16–18). Apparent rates of leucine appearance and disappearance calculated from [$^{2}H_3$]leucine were used to determine leucine carbon (C) kinetics, since this tracer provides rates of leucine flux similar to those obtained with [U-^{14}C]leucine (21), [1-^{14}C]leucine (36), and [1-^{13}C]leucine (37). Rates of appearance of [^{15}N]alanine, percentage of alanine nitrogen derived from leucine, and the percentage and rate of leucine nitrogen transfer to alanine were calculated using standard product precursor relationships (16–18).

Results

Plasma hormone concentrations. During the infusion of hydrocortisone, plasma cortisol concentrations increased ($P < 0.001$) from 10±1 μg/dl to 42±4 μg/dl by hour 3, and subsequently remained nearly constant. During infusion of saline, plasma cortisol concentrations decreased from 14±2 μg/dl to 9.5±2 μg/dl ($P < 0.05$) at hour 8 (Fig. 1). Plasma concentrations of insulin (9.0±0.2 μU/ml and 9.1±0.2 μU/ml), C-peptide (1.7±0.1 and 1.9±0.1 μg/ml), and glucagon (287±5 and 298±12 pg/ml) were not significantly different before infusion of saline or cortisol and did not change subsequently (Fig. 1).

Effects of cortisol on leucine metabolism. Plasma leucine

1. *Abbreviations used in this paper:* GC/MS, gas chromatography-mass spectrometry; KIC, α-ketoisocaproate; Ra, rate(s) of appearance; Rd, rate(s) of disappearance.

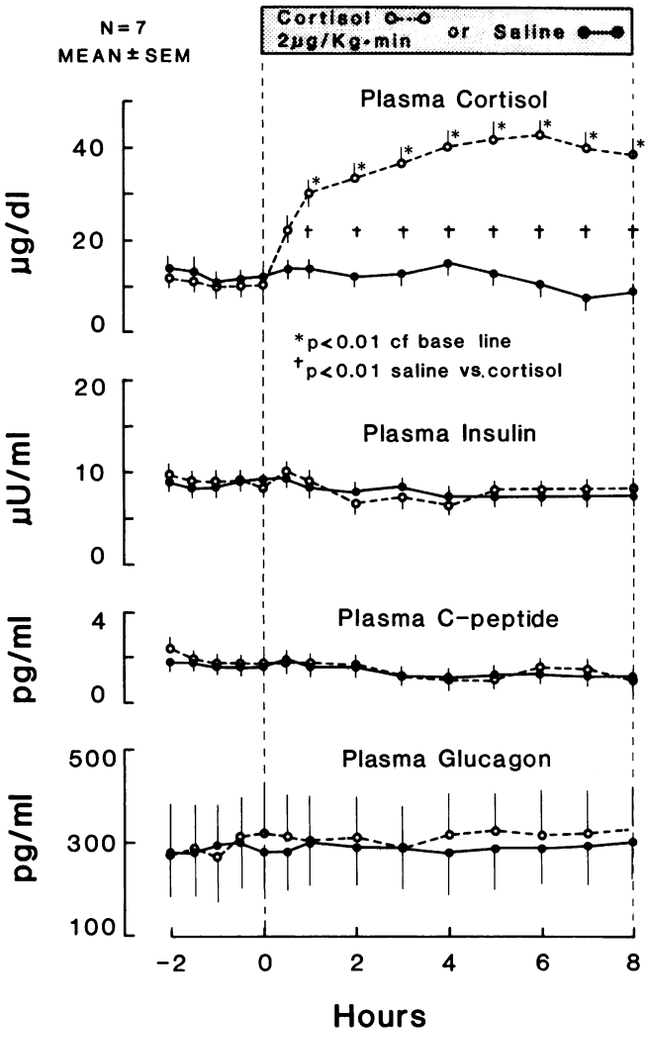

Figure 1. Plasma cortisol, insulin, C-peptide, and glucagon concentration during saline (•) or cortisol (○) infusion in seven normal adults.

concentration increased 70% (120±1 to 203±21 μM, $P < 0.01$) during cortisol, but only 30% (118±8 to 154±4 μM, $P < 0.05$) during saline infusion. Although base-line leucine values were not different on the two study days, plasma leucine concentration was higher ($P < 0.05$) by hour 3 of the cortisol when compared with that of the saline infusion and remained so for the duration of study (Fig. 2).

During cortisol infusion, the rate of leucine carbon (C) appearance increased ($P < 0.01$) by 23% from 1.47±0.08 to 1.81±0.08 μmol/kg·min; whereas during saline infusion leucine C Ra did not change (1.50±0.08 vs. 1.57±0.09 μmol/kg·min) (Fig. 2). The Rd of leucine carbon also increased ($P < 0.01$) by 24% from 1.47±0.05 to 1.83±0.09 μmol/kg·min during the cortisol infusion but remained unchanged during the saline infusion (1.50±0.08 vs. 1.55±0.05 μmol/kg·min) (Fig. 2).

The Ra of leucine nitrogen (N) increased ($P < 0.01$) by nearly 50% from 2.35±0.12 to 3.46±0.24 μmol/kg·min during the cortisol infusion, but increased by only 17% ($P < 0.05$) during the saline infusion (2.43±0.17 to 2.85±0.15 μmol/kg·min) (Fig. 3). The increase in leucine N Ra during cortisol administration was greater ($P < 0.01$) than that observed during saline infusion. The Rd of leucine N increased ($P < 0.01$) by 50% during cortisol infusion (2.35±0.12 to 3.48±0.25 μmol/kg·min) but by only 17% ($P < 0.05$) during the saline infusion (2.43±0.17 to 2.84±0.15 μmol/kg·min) (Fig. 3).

Effect of cortisol on alanine metabolism. For unexplained reasons, plasma alanine concentrations and alanine Ra and Rd after an overnight fast were different ($P < 0.01$) on the two days of study. During the cortisol infusion, plasma alanine concentrations did not change (184±19 to 187±21 μM at hour 8). In contrast, during the saline infusion plasma alanine decreased ($P < 0.01$) from 278±22 to 214±11 μM. Alanine Ra during

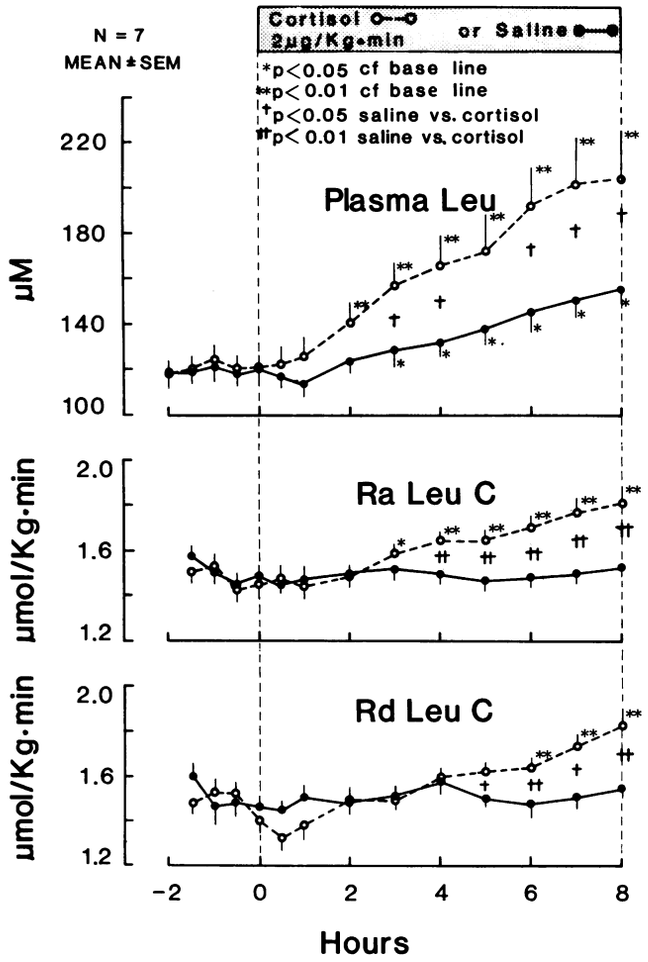

Figure 2. Plasma leucine (leu) concentration and Ra and Rd of leucine carbon (C) as estimated by [$^2H_3$] leucine infusion in seven normal volunteers during saline (•) or cortisol (○) infusion.

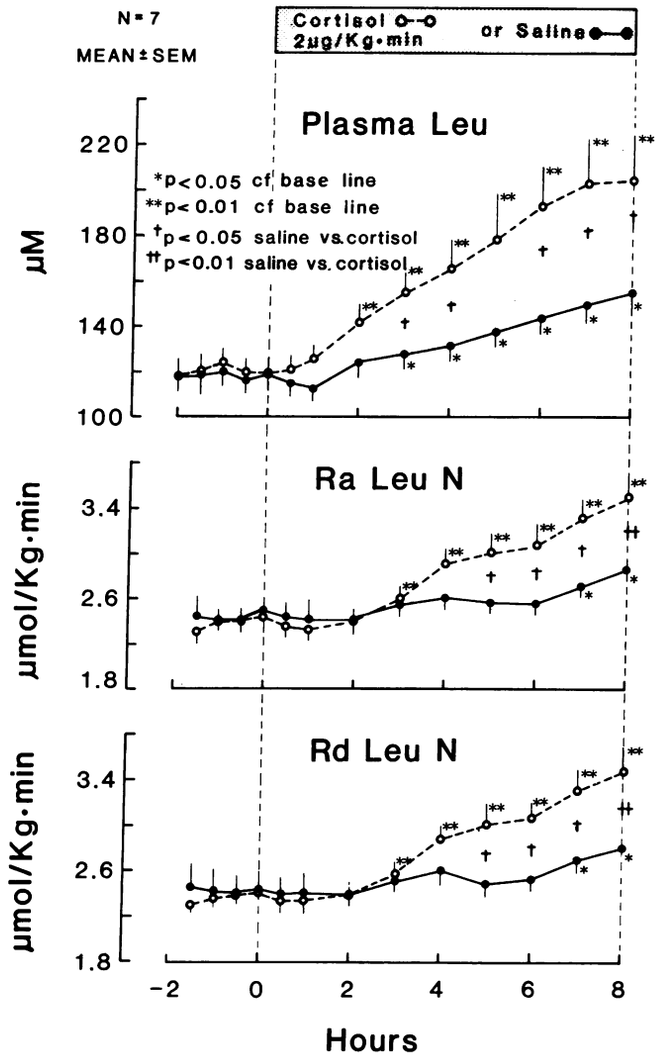

Figure 3. Plasma leucine (leu) concentration and Ra and Rd of leucine nitrogen (N) in seven normal adults during saline (●) or cortisol (○) infusion.

the cortisol infusion increased ($P < 0.05$) from 4.56±0.23 to 6.29±0.52 μmol/kg·min by hour 8, whereas during saline infusion, it did not change (5.91±0.79 to 5.31±0.44 μmol/kg·min by hour 8). Alanine Rd increased ($P < 0.05$) during the cortisol infusion (4.57±0.24 to 6.28±0.51 μmol/kg·min by hour 8). During saline infusion, alanine Rd did not change and values were similar to those of alanine Ra.

Since the base-line values for plasma alanine concentration were different on the two study days, these data are plotted as absolute change from the mean basal values to facilitate comparison between those of saline and cortisol infusion studies (Fig. 4). Following 1 h of saline infusion, the plasma alanine concentrations were lower ($P < 0.05$) than the basal values (Δ −32±12 μM). By hour 8, the plasma alanine had decreased ($P < 0.01$) by 64±17 μM. In contrast, during cortisol infusion, plasma alanine did not significantly change (Δ −9±11 μM at hour 8, cf basal values). The change in plasma alanine concentration between the two study days was different ($P < 0.05$) between hour 7 and 8 (Fig. 4). Alanine Ra and Rd decreased during the saline infusion but were not significantly different from basal values. In contrast, during cortisol infusion, alanine Ra and Rd were increased ($P < 0.05$) above basal values by hour 7; and, the Ra and Rd of alanine between the infusion days were different ($P < 0.05$) from hour 5 to the end of the study (Fig. 4).

Effect of cortisol on leucine and alanine nitrogen interrelationships. The basal rate and percentage of leucine N transfer to alanine N were greater ($P < 0.05$) on the day of saline (0.93±0.13 μmol/kg·min and 40±5%, respectively) when compared with the day of cortisol infusion (0.70±0.03 μmol/kg·min and 30±2%, respectively); whereas the percentage of alanine N

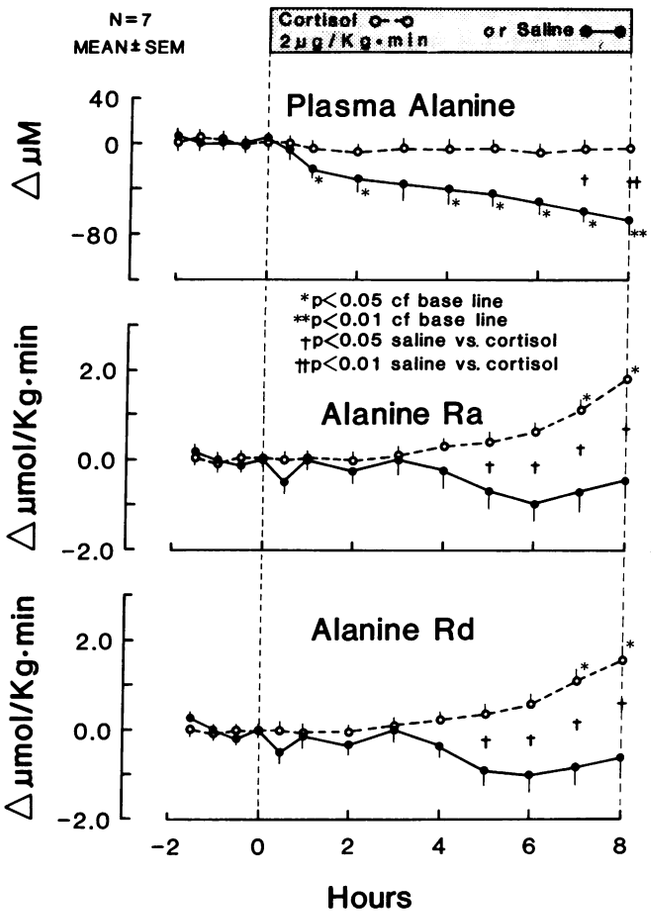

Figure 4. The change in plasma alanine concentration and the change in the rate of alanine Ra and Rd in seven normal adults during saline (●) or cortisol (○) infusion.

derived from leucine N was similar on the two study days (15±2 vs. 16±1%, respectively). Because of the differences in basal values, the data are expressed as change from base line to facilitate comparison between the saline and cortisol studies (Fig. 5).

During cortisol infusion, the rate of leucine N transferred to alanine N was increased ($P < 0.05$) above the basal value during the last 4 h of study. The increase was greater over the last 3 h of study ($P < 0.02$) than that observed during saline infusion, which did not change over the course of the study. The percentage of leucine N transferred to alanine increased ($P < 0.05$) at hour 7 and 8 during only the cortisol infusion, and was significantly different from that of the saline infusion at hour 8 (Fig. 5). An increase ($P < 0.02$) in the percentage of alanine nitrogen derived from leucine was observed over the last 3 h during both the cortisol and saline infusions.

Effects of cortisol infusion on the plasma concentration of other amino acids. The summed total of all the plasma amino acid concentrations was increased ($P < 0.05$) during the cortisol infusion but not during saline infusion (Table I). This is primarily attributable to the increase in the plasma concentrations of valine, leucine, isoleucine, tyrosine, phenylalanine, and histidine during cortisol but not during saline infusion.

Glucose kinetics. Glucose kinetics determined by infusion of [2-^{3}H]glucose in three subjects or [3-^{3}H]glucose in four subjects were similar during infusions of cortisol or saline. As a result, these data were pooled. The small but significant increase ($P < 0.01$) in plasma glucose concentration (89±2 to 94±2 mg/dl) during cortisol infusion was not the result of an increase in glucose Ra (2.1±0.1 to 1.9±0.1 mg/kg·min at 0 and 8 h, respectively). Therefore, the increased plasma glucose must have resulted from the relative decrease in glucose Rd as reflected by the reduction of ($P < 0.02$) in glucose metabolic clearance rate (2.34±0.09 to 1.96±0.16 ml/kg·min) during cortisol infusion (Table II). During saline infusion, plasma glucose concentrations decreased ($P < 0.01$) from 90±1 to 81±2 mg/dl as a result of a decrease ($P < 0.01$) in glucose Ra (2.2±0.2 to 2.0±0.1 mg/kg·min).

Effect of cortisol on plasma pyruvate, lactate, FFA, and ketone body concentrations. Plasma pyruvate concentrations increased over the course of both study days, whereas plasma lactate con-

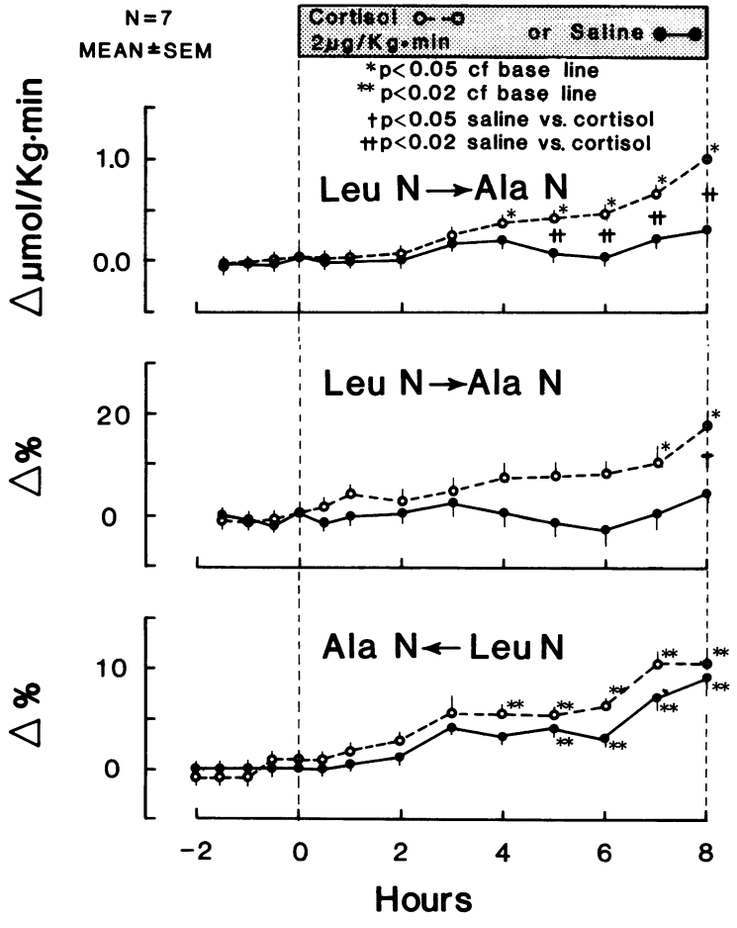

Figure 5. The change in the rate of leucine nitrogen transferred to alanine (Leu N → Ala N), the change in percentage of leucine nitrogen going to alanine nitrogen (Leu N → Ala N), and the change in percentage of alanine nitrogen derived from leucine nitrogen (Ala N ← Leu N) in seven normal adults during saline (●) or cortisol (○) infusion.

Table I. Plasma Amino Acid Concentrations during Saline and Cortisol Infusions in Normal Humans

		−60	0	240	480
Total	Saline	1,692±73	1,673±78	1,623±96	1,602±80
	Cortisol	1,534±119	1,466±85	1,547±102	1,694±63*
Threonine	Saline	117±10	113±11	104±12	96±11
	Cortisol	105±19	102±17	91±15	85±11
Serine	Saline	133±3	130±2	125±4	119±5
	Cortisol	122±11	121±10	107±10	105±9
Asparagine	Saline	43±3	42±2	41±5	35±2
	Cortisol	40±5	37±3	36±3	42±4
Glutamic acid	Saline	55±3	52±4	50±4	40±4
	Cortisol	59±6	54±4	48±5	40±4
Glycine	Saline	272±31	270±30	254±27	240±24
	Cortisol	234±26	230±24	204±25	194±21
Alanine	Saline	277±24	281±19	239±14	214±11
	Cortisol	193±20	192±15	177±16	174±21
Valine	Saline	165±14	157±11	160±10	166±22
	Cortisol	165±9	143±15	191±14*	231±27*
Isoleucine	Saline	35±5	39±4	40±5	51±6
	Cortisol	37±3	34±2	56±6*	85±8*
Leucine	Saline	120±9	119±8	132±8	154±9*
	Cortisol	125±5	119±7	167±17*	203±21*
Tyrosine	Saline	37±3	32±2	34±4	34±3
	Cortisol	30±3	29±2	30±2	36±2*
Phenylalanine	Saline	46±6	42±4	44±3	46±5
	Cortisol	40±4	38±4	46±4	53±3*
Ornithine	Saline	85±10	80±9	82±4	86±12
	Cortisol	71±8	68±8	70±8	72±7
Lysine	Saline	187±17	184±17	182±21	184±21
	Cortisol	178±20	169±19	177±20	183±17
Arginine	Saline	84±6	85±6	83±9	77±7
	Cortisol	72±8	73±9	74±9	78±9
Histidine	Saline	78±4	79±5	81±4	82±4
	Cortisol	78±5	76±4	82±4	85±4*

Units are micromolar.
* $P < 0.05$ from base line.

centrations did not change during cortisol infusion but decreased during saline infusion ($P < 0.05$) (Table II). Plasma FFA, β-hydroxybutyrate, and acetoacetate concentrations increased similarly on both study days (Table II).

Discussion

The present study, using isotope dilution techniques, demonstrates that an increase in plasma cortisol within the physiologic range increases the plasma leucine concentration as a result of a greater stimulation of the Ra than Rd of leucine. The increase in the leucine Ra occurred in the absence of changes in plasma insulin, C-peptide, and glucagon. Since the only source of this essential amino acid in the postabsorptive state is tissue protein, we conclude that an increase of plasma cortisol within the physiologic range stimulates whole body proteolysis. This conclusion is consistent with the observed increased urinary nitrogen excretion (14) and with elevations in the plasma concentrations of other amino acids in this and previous studies (13–15). The tissue(s) in which this increased protein breakdown occurs cannot be determined from our studies. Pharmacologic doses of glucocorticosteroids result in increased 3-methylhistidine excretion (8), suggesting that proteolysis in skeletal muscle may be enhanced. Whether a physiologic increase in plasma glucocorticoid concentrations has similar effects is not known. However, cortisol may affect proteolysis to a greater extent in tissues with more rapid protein turnover (e.g., liver, gut, skin, bone marrow) than in skeletal muscle (38).

Due to the relatively long period of isotope infusion in our study, labeled leucine may have been incorporated into and released from protein (39). This potential recycling of label would

Table II. Plasma Substrate Concentrations and Glucose Kinetics during Cortisol or Saline Infusions in Normal Humans

Time (hours):		−2	0	4	8
Glucose					
Concentration (*mg/dl*)	Saline	93±2	90±1	84±2*	81±2‡
	Cortisol	89±1	98±2	92±2*§	94±2‡‖
Ra (*mg/kg · min*)	Saline	2.2±0.2	2.2±0.2	2.2±0.2	2.0±0.1‡
	Cortisol	2.1±0.1	1.9±0.1	1.9±0.1	1.9±0.1
Rd (*mg/kg · min*)	Saline	2.2±0.2	2.2±0.2	2.1±0.1	2.0±0.1
	Cortisol	2.1±0.1	2.0±0.1	1.9±0.1	1.9±0.1‡
Clearance (*ml/kg · min*)	Saline	2.45±0.19	2.43±0.19	2.55±0.15	2.49±0.13
	Cortisol	2.34±0.09	2.23±0.13	2.06±0.12	1.96±0.16¶§
Lactate (*μM*)	Saline	940±80	840±80	670±40*	670±70*
	Cortisol	840±30	730±50	730±30	730±60
Pyruvate (*μM*)	Saline	35±11	44±12	51±14	82±10*
	Cortisol	46±12	55±131	60±7	85±5‡
FFA (*μM*)	Saline	810±120	830±100	890±70	1060±90‡
	Cortisol	530±100	650±60	830±90	990±110¶
β-Hydroxybutyrate (*μM*)	Saline	200±100	320±100	410±110‡	870±160‡
	Cortisol	210±41	280±46	600±100‡	1070±140‡
Acetoacetate (*μM*)	Saline	150±50	170±50	220±50*	420±50‡
	Cortisol	120±20	160±30	320±30‡	470±40‡
Total ketone bodies (*μM*)	Saline	450±160	490±150	720±154‡	1300±210‡
	Cortisol	330±60	440±66	910±100‡	1540±140‡

* $P < 0.05$ cf base line.
‡ $P < 0.01$ cf base line.
§ $P < 0.05$ cf base line.
‖ $P < 0.01$ cf saline.
¶ $P < 0.02$ cf base line.

result in an underestimation in the Ra of leucine during both saline and cortisol infusions. Therefore, it is possible that the cortisol-induced increase in proteolysis observed at 3 h may have occurred even earlier and/or to a greater extent. Thus, our calculations may represent an underestimation of the effect of cortisol on proteolysis in man.

In addition to an increase in the Ra of leucine, cortisol infusion increased the Rd of leucine. Leucine released from body protein can be either utilized for protein synthesis or undergo irreversible oxidative decarboxylation following transamination to its α-ketoacid, α-ketoisocaproate (KIC). High dose in vivo glucocorticoid treatment inhibits protein synthesis in rat skeletal muscle (9–12), but its effect on nonskeletal muscle protein is not known. If glucocorticoids not only stimulate proteolysis, as the present studies demonstrate, but also inhibit protein synthesis, then the rate of leucine oxidation would have to increase dramatically. This would lead to accelerated loss of an essential amino acid and ultimately to severe protein depletion. Although glucocorticoid excess results in negative protein balance, major protein loss occurs only after weeks of exposure to pharmacologic levels. Therefore, the increase in leucine utilization may reflect some stimulation of leucine oxidation, but most likely represents an increase in protein synthesis in some body tissues as well. Clarification of the ultimate fate of leucine released from body protein awaits the measurement of leucine oxidation under similar experimental conditions.

As demonstrated here and in previous studies in man and dogs (16–18, 40), the flux of leucine nitrogen, as estimated from the [^{15}N]leucine data, exceeds that of leucine carbon. Since [$^{2}H_3$]leucine is probably transaminated to KIC and back to leucine without loss of label whereas the ^{15}N would be lost to a large nitrogen pool, these results are consistent with a rate of leucine transamination that is higher than irreversible loss of leucine carbon. Whether this high rate of transamination reflects "futile" transamination of leucine in a single tissue or its transport as the α-ketoacid from peripheral tissues to liver under these conditions remains unknown; however, recent studies in the rats (41) and dogs (42) lend support to the latter hypothesis. If the ratio of leucine N to leucine C flux is a reflection of the activity of the proposed leucine-KIC shuttle, then cortisol has no effect on the relative activity of this shuttle since the changes in the ratio of the leucine N/leucine C flux were identical during cortisol and saline infusions (1.47–1.80 with saline; 1.47–1.89 with cortisol).

In the postabsorptive state, alanine appearance into the plasma space represents the sum of alanine release from protein (proteolysis) and production from *de novo* synthesis (16–18, 43). Assuming alanine and leucine content of body protein are 6 and 8%, respectively (44), the rate of alanine release from protein can be calculated from leucine C appearance rates (16–18, 43). The calculated rate of alanine release from protein during cortisol infusion increased 23% (from 1.63 to 2.00 μmol/kg · min), whereas during saline infusion, it increased by only 5% (from 1.66 to 1.74 μmol/kg · min). The total alanine Ra increased from 4.56 to 6.29 μmol/kg · min during cortisol infusion; therefore, the rate of alanine synthesis (alanine Ra − alanine released from protein) increased by 46% from 2.93 to 4.29 μmol/kg · min. In contrast, alanine synthesis during saline infusion did not increase (from 4.25 to 3.57 μmol/kg · min). The fact that the rate and percentage of leucine N going to alanine significantly increased only during cortisol infusion is further evidence of a cortisol-mediated stimulation of *de novo* alanine synthesis. Because the increases in the percentage of alanine N derived from leucine were not different on the two study days, we conclude that cortisol did not selectively affect the transfer of N from the branched-chain amino acids to alanine. It is of interest to note that the difference in basal alanine flux on the two study days is attributable to differences in the rates of alanine synthesis, rather than of proteolysis, since basal leucine carbon rates of appearance were similar on the two study days. This interpretation is supported by our observation of higher basal rates and percentages of leucine N transfer to alanine during the base-line period of study on the saline infusion day as compared with the day of cortisol infusion.

During cortisol infusion alanine Ra increased. However, alanine concentration did not change because its Rd increased to the same extent. In contrast, the plasma alanine concentration during saline infusion decreased. This was presumably due to a decrease in alanine Ra, since alanine Rd did not increase. It should be noted that the changes in the plasma concentration of lactate, a potential carbon source for alanine synthesis, paralleled those of alanine on the two study days. However, plasma concentrations of pyruvate, the immediate precursor to alanine synthesis, did not follow this pattern. During cortisol infusion, the sustained plasma lactate and alanine concentrations and the increase in alanine turnover were associated with higher glucose concentrations than observed on the control day. Whether this higher plasma glucose concentration was the result of increased availability of these gluconeogenic substrates cannot be determined from the present studies, since a carbon-labeled alanine was not used.

In summary, the present studies demonstrate that an increase of cortisol within the physiologic range stimulates proteolysis independently of changes in plasma insulin and glucagon. The increase in alanine turnover observed during cortisol infusion was not only the result of enhanced proteolysis but of an even greater increase in alanine synthesis. The latter is consistent with a cortisol-mediated stimulation of the postulated glucose-alanine cycle (45). The increased availability of potential gluconeogenic substrates (alanine and possibly lactate and pyruvate) may contribute to the changes in carbohydrate metabolism observed during stress and other hypercortisolemic states.

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